Cyanogenesis in Plants.

Part IV.—*The Occurrence of Phaseolunatin in Common Flax* (Linum usitatissimum).

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In Part III of this series of papers* it was shown that the seeds of *Phaseolus lunatus*, as produced by the uncultivated plant in Mauritius, contained a new cyanogenetic glucoside, phaseolunatin, which was proved to have the constitution of a dextrose ether of acetonecyanhydrin. Phaseolunatin was further shown to undergo hydrolysis by mineral acids or by the action of the characteristic, emulsin-like enzyme also present in the seeds, yielding, as final products, acetone, hydrocyanic acid, and dextrose.

This decomposition of the glucoside by the enzyme takes place when the ground seeds of *Phaseolus lunatus* are mixed with water, and from such a preparation it is comparatively easy to isolate and identify the hydrolytic products, acetone and hydrocyanic acid. The simultaneous production, therefore, of these two substances from plants by mere contact with water may be taken as indicating the occurrence in such plants of phaseolunatin or some similar derivative of acetonecyanhydrin, and of an enzyme capable of decomposing this compound under the conditions specified.

Before the isolation of phaseolunatin, it was observed by Van Romburgh† that various plants, on crushing with water and subsequent distillation, yielded acetone, and that in some cases this was accompanied by hydrocyanic acid. The plants mentioned by Van Romburgh as yielding both these products are *Phaseolus lunatus*, *Manihot utilissima* (the Cassava plant from which the tapioca of commerce is prepared), *Manihot glaziovii* (the Ceara rubber tree), and *Hevea brasiliensis* (the Para rubber tree). This author stated that he was at first inclined to associate the simultaneous production of acetone and hydrocyanic acid from these plants with the occurrence in them of a compound of these two substances, but his subsequent discovery that several plants, notably *Erythroxylon Coca*, from which the "Coca leaves," largely used as a stimulant in South America, are procured, and *Pogostemon cristatus*, yielded acetone unaccompanied by hydrocyanic acid, led him to

* 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

† 'Annales du Jardin Botanique de Buitenzorg,' 1899, vol. 2, 1, p. 2.

abandon this idea, and he suggested that the acetone might occur in the form of a glucoside.

Quite recently Van Itallie has observed* that the leaves of *Thalictrum aquilegifolium* yield acetone and hydrocyanic acid under similar conditions, and he has suggested that this plant may contain the glucoside phaseolunatin which we had isolated previously from the seeds of *Phaseolus lunatus*.

A systematic investigation of these various plants is being made, as a part of the general work on cyanogenesis we have undertaken, with a view to ascertaining definitely whether they contain phaseolunatin, and in the present and succeeding papers two cases are dealt with, namely, the "flax" and "cassava" plants. The seeds of the Para rubber tree are at present under investigation, and the examination of the other plants mentioned will be commenced as soon as material, which is rather difficult to procure, becomes available.

Cyanogenesis in flax was first observed by Jorissen,† who stated (1) that, when linseed meal (ground flax seed) is allowed to stand with warm water at 25° C., hydrocyanic acid is produced, and may be obtained by distillation of the mixture, and (2) that the acid does not exist preformed in the seed, since the latter does not yield it when placed in boiling water. This author suggested that flax seed probably contains a substance on which emulsin acts in the same way as on amygdalin, and further noted that linseed meal has the property of decomposing amygdalin, liberating benzaldehyde, hydrocyanic acid, and dextrose. In subsequent papers, Jorissen stated that both *Linum usitatissimum* and *Linum perenne* contain amygdalin in the leaves and stems, and that, in the case of the former, a notable increase in the amount of hydrocyanic acid obtainable takes place on germination of the seed. Thus, whereas a portion of one sample of flax seed yielded 0·01 per cent. of the acid, another portion of the same sample, after germination, yielded 0·07 per cent.‡

Subsequently, Jorissen and Hairs§ succeeded in isolating the cyanogenetic glucoside of flax in a crystalline condition. They named it "linamarin," and described it as crystallising in groups of colourless needles, melting at 134° C., having a cool and bitter taste, and being readily soluble in water.

They assigned no formula to linamarin, but stated that, on combustion, it gave the following results: carbon, 47·88 per cent.; hydrogen, 6·68 per cent.; oxygen, 39·89 per cent.; and nitrogen, 5·55 per cent.; and that, on hydro-

* 'Journ. Pharm. Chim.,' 1905, vol. 6, pp. 22, 337.
† 'Bull. Acad. Roy. Belg.,' 1883 (iii), vol. 5, p. 750.
‡ *Loc. cit.*, 1884 (iii), vol. 6, p. 718, and vol. 7, p. 736.
§ *Loc. cit.*, 1891 (iii), vol. 21, p. 529.

lysis, by heating with dilute mineral acids, or by the addition of linseed meal to an aqueous solution of the glucoside, the latter was decomposed, yielding hydrocyanic acid, a reducing sugar, and a volatile ketone, which gave the iodoform reaction. Jorissen and Hairs also examined the enzyme contained in embryonic flax plants, and observed that, whilst it had the property of hydrolysing both linamarin and amygdalin, the emulsin of almonds was incapable of decomposing linamarin.

Van de Ven, who attempted to repeat the work of Jorissen and Hairs, using flax seed as a source of the glucoside, did not succeed in isolating linamarin, and he was also unable to find that hydrocyanic acid could be obtained from the seed.* Jouck, however,† was able to confirm and extend Jorissen and Hairs' observations, and identified the volatile ketone produced by the hydrolysis of the flax glucoside as acetone. This author, however, found, in opposition to Jorissen and Hairs, that linamarin is decomposed by the emulsin of almonds.

Preliminary Experiments.

In the present investigation, attempts were first made to utilise flax seed cake (linseed cake) as a raw material for the isolation of the glucoside, but it was found that, although distinct evidence of the presence of a cyanogenetic glucoside in the cake was readily obtainable, the quantity present was so small that it was impossible to isolate it in a crystalline condition. It was considered advisable, therefore, to have recourse to the use of young flax plants as a source of linamarin. The young flax plants for the investigation were grown during the summer of 1905, partly at the Imperial Institute and partly by permission of Professor J. B. Farmer, F.R.S.—to whom we are indebted for the help thus rendered—at the Chelsea Physic Garden. Small supplies of immature flax were also received during the earlier part of the investigation from Mr. F. Barbour, of the Department of Agriculture and Technical Instruction in Ireland, to whom our thanks are also due.

As a preliminary measure, the amount of glucoside present in whole flax plants, including roots, at various stages of growth was determined, with a view to the selection of the richest material. This estimation was carried out by completely extracting a weighed quantity of the air-dried plant with alcohol, distilling the solvent from the extract, dissolving the residue in water, hydrolysing the glucoside contained in this by boiling with hydrochloric acid, and finally distilling off and estimating the hydrocyanic acid in the

* Van Rijn, 'Die Glykoside,' 1900.

† 'Beitrag zur Kenntnis der Blausäure abspaltenden Glycoside,' Inaug. Diss., Strassburg, 1902.

distillate. Detailed accounts of the methods of carrying out the estimation of the amounts of cyanogenetic glucoside contained in plants are given in the preceding papers of this series.*

The results obtained by the estimation of the amounts of hydrocyanic acid yielded under these conditions by young flax plants were as follows:—

Height of flax plants in inches.	Grown at—	Hydrocyanic acid found.	Glucoside calculated.
		per cent.	per cent.
Seed	—	0·008	0·07
1— 1·5	Imperial Institute	0·15	1·4
2— 3	,, ,,	0·17	1·5
3— 4	,, ,,	0·15	1·4
4— 5	Physic Garden, Chelsea	0·13	1·2
5— 6	,, ,, ...	0·10	0·9
6— 7	,, ,, ...	0·10	0·9
8—10	,, ,, ...	0·08	0·7
12—15	,, ,, ...	0·07	0·6
15—18	,, ,, ...	0·03	0·3
18	,, ,, ...	0·009	0·08
18	,, ,, ...	None	None

These results are of special interest since, apart from indicating the stages in the growth of the plant at which the isolation of the glucoside could be most hopefully undertaken, they show that the course of cyanogenesis in the flax plant is different from that in *Lotus arabicus* and in *Sorghum vulgare*. In each of these two plants it has been shown in the first two papers of this series† that the amount of the characteristic glucoside present steadily increases until the plants approach maturity, then decreases, and that none is present in the seed. In flax, on the other hand, the seed contains a small amount of the glucoside which increases on germination, reaches a maximum at a very early stage in the growth of the plant (when it is from 2 to 3 inches high) and then diminishes steadily and finally disappears altogether. These results on the whole confirm those of Jorissen, who also observed that a very large increase of the glucoside occurred with the germination of the seed, though the amounts recorded by him are smaller than those now found. These differences are probably due to the fact that Jorissen estimated the amount of glucoside present by determining the amount of hydrocyanic acid formed, by merely moistening a known weight of ground seed. Under these conditions it is improbable that the whole of the glucoside undergoes hydrolysis, and for this reason Jorissen's results are probably too low.

* Dunstan and Henry, 'Phil. Trans.,' B, 1901, vol. 194, p. 515; A, 1902, vol. 199, p. 399; and 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

† *Loc. cit.*

Isolation of the Glucoside.

Flax plants (stems, leaves and roots) of about 4 or 5 inches in height were thoroughly dried at a temperature not exceeding 40° C. The dried material was ground to a fine powder and completely extracted with 90 per cent. alcohol. The greater part of the solvent was then distilled off and the residue poured into water, whereby chlorophyll, oily and resinous matters, were precipitated. The aqueous liquid after decantation was decolorised by the addition of a slight excess of lead acetate, and the filtrate, after treatment with sulphuretted hydrogen, to remove the excess of lead, was evaporated down to a low bulk under reduced pressure and set aside for a time. This purified and decolorised extract was very rich in the cyanogenetic glucoside, but it could not be induced to crystallise out of the syrupy mass. The latter was, therefore, redissolved in alcohol and the solution poured into six times its volume of ether. The sticky precipitate which separated consisted principally of a mixture of dextrose and potassium nitrate. The solvent was distilled from the decanted liquid and the dry residue again dissolved in alcohol and the precipitation by means of excess of ether repeated. In this case the precipitate was principally dextrose with a small quantity of the glucoside. The solvent was again distilled from the decanted liquid and the light brown syrupy residue set aside. After some days, masses of needles arranged in rosettes began to separate and in a short time the whole of the syrup had solidified, forming a crystalline mass. This was spread on a recently-ignited porous tile and the fairly clean crystals, thus separated from the viscous mother liquor, dissolved in alcohol and recrystallised until they were colourless and of constant melting point. This material was carefully compared with phaseolunatin prepared from the seeds of *Phaseolus lunatus* and the two substances were proved to be identical.

Determinations of the melting points of phaseolunatin and the flax glucoside under the same conditions gave 138° C. (corr.) as the melting point of each, and a mixture of the two substances also melted at this temperature.

The flax glucoside was found to have the specific rotation $[\alpha]_D$ — 27°·4 in alcohol at 15°; that previously recorded for phaseolunatin is — 26°·2 under the same conditions.

Finally both substances crystallise in the same characteristic, spreading rosettes of slender needles, and possess the same peculiar cool and bitter taste. The total quantity of the glucoside obtained by us from flax did not amount to more than 0·3 gramme, and as the results of the comparison of its physical constants with those of phaseolunatin so positively established the

identity of the two substances, we considered it inadvisable to use the considerable proportion of the whole of our material, which would have been necessary, in making a combustion. The average of the combustion results quoted by Jorissen and Hairs for the flax glucoside agrees closely with those required by the formula assigned in the preceding paper of this series to phaseolunatin, viz.:—

Found by Jorissen and Hairs for linamarin—

C = 47·88 per cent.
H = 6·68 „
O = 39·89 „
N = 5·55 „

Required for phaseolunatin ($C_{10}H_{17}O_6N$)—

C = 48·1 per cent.
H = 6·8 „
O = 39·5 „
N = 5·6 „

Hydrolytic Products of the Flax Glucoside.

It has already been pointed out that Jorissen and Hairs observed that the flax glucoside was decomposed by boiling with dilute acids, liberating a reducing sugar, hydrocyanic acid and a volatile ketone. The latter was subsequently identified by Jouck as acetone. For the examination of the volatile hydrolytic products of the flax glucoside we have used a portion of the purified extract from which the glucoside was eventually induced to crystallise. The purified extract was dissolved in water, and a few cubic centimetres of 10-per-cent. hydrochloric acid added; this liquid was then distilled almost to dryness. The distillate had a strong odour of hydrocyanic acid and gave the Prussian blue reaction copiously. The remainder of the distillate was then rendered alkaline and redistilled, the first few cubic centimetres being collected separately. To this were added a few drops of benzaldehyde and a small quantity of an aqueous solution of potassium hydroxide. On standing, this mixture deposited crystals which on recrystallisation melted at 112° C., and proved to be identical with dibenzylideneacetone (melting point, 112° C.). The second volatile hydrolytic product is therefore acetone. The identity of the volatile hydrolytic products of the flax glucoside with those of phaseolunatin affords a further proof that the cyanogenetic glucoside of flax is phaseolunatin.

There seems to be no reason therefore why the name linamarin applied

by Jorissen and Hairs to the cyanogenetic glucoside of flax should be retained.

Other Constituents of Flax.

It has already been mentioned that a considerable amount of potassium nitrate was found to have accumulated in the purified extract from which the flax glucoside eventually crystallised.

This occurrence of potassium nitrate with the cyanogenetic glucoside in flax is of some significance, since Treub* has pointed out that the accumulation of potassium nitrate in the stems and petioles of *Phaseolus lunatus* has an intimate connection with the secretion of phaseolunatin by that plant, and has suggested that this store of nitrate may be the raw material from which the cyanogenetic glucoside in this plant is eventually produced.

From one of the purified extracts, obtained from flax 12 inches high, grown in Ireland, a small quantity of a sugar crystallising in characteristic, cauliflower-like masses separated after the extract had stood for some time. It melted at 78° to 80° after recrystallisation from alcohol, did not reduce Fehling's solution, and was slightly dextrorotatory. When an aqueous solution was boiled with mineral acids a reducing sugar was produced. These observations seem to indicate that this material may be identical with raffinose (melting point 80° C.), but a sufficient quantity of the sugar could not be obtained for complete examination.

The Enzyme of Flax.

Preparations of this were made by macerating finely-ground flax seed (linseed) with water, previously saturated with chloroform to render the liquid antiseptic. This extract was found to have a range of activities similar to that of the emulsin of almonds, and it readily hydrolysed amygdalin and salicin. When added to an aqueous solution of the purified flax extract, prepared as already described, it speedily hydrolysed the contained phaseolunatin, yielding acetone and hydrocyanic acid, which were identified in the usual way.

Preparations of the enzyme were also found to hydrolyse phaseolunatin prepared from the seeds of wild *Phaseolus lunatus* and, *vice versâ*, preparations of the glucosidolytic enzyme contained in seeds of *Phaseolus lunatus* were found to hydrolyse the phaseolunatin obtained from flax seed, the volatile hydrolytic products being in both cases the same, viz., acetone and hydrocyanic acid.

It seems probable, therefore, that the same enzyme is contained both

* 'Ann. Jard. Bot. de Buitenzorg,' 1905, vol. 2, 4, p. 86.

in *Phaseolus lunatus* seed and in flax seed. This enzyme is of the emulsin type (*i.e.*, it appears to hydrolyse β-glucosides) and exhibits similar activities, but it also presents certain well-marked differences from emulsin, which will be the subject of further investigation.

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Part V.—*The Occurrence of Phaseolunatin in Cassava* (Manihot Aipi *and* Manihot utilissima).

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The "sweet" and "bitter" cassava plants are indigenous to Southern and Central America, whence they have been introduced, especially the sweet variety, into almost all tropical countries and are now widely cultivated for the sake of their edible starchy roots, which are used for the manufacture of the various forms of cassava starch, of which tapioca is perhaps the best known.

The plants are known in their native habitat by a variety of vernacular names of which only one, "manioc" or "mandioca," has come into general use. The name "cassava" seems to be restricted in South America to the flour or meal made from the roots, but outside South America this name has come to be applied to the whole plant.

There are many varieties of cassava plants in cultivation in the tropics, but these all appear to belong to either the "bitter" or "sweet" forms. These two forms were regarded by Pohl* as distinct species and were named by him *Manihot utilissima* and *Manihot Aipi* respectively.

By other botanists the "sweet" cassava is regarded as a variety or perhaps a cultivated race of *Manihot utilissima*,† whilst others take the view that Pohl's *Manihot Aipi* is identical with *Manihot palmata*.‡ Colonel Prain, Director of the Royal Gardens, Kew, whom we have consulted on this point, is of opinion that on the evidence at present available, Pohl's view,

* 'Pl. Bras. Ic.,' i, vol. 32, p. 24.

† Compare Sagot, 'Bull. Soc. Bot. France,' 1872, vol. 18, p. 341.

‡ 'Index Kewensis,' fasc. iii, p. 162; and Peckolt, 'Pharm. Rund.,' 1886, vol. 4, p. 57.